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TITLE:
Developing Treatment, Treatment Validation, and Treatment Scope in the Setting of an Autism Clinical Trial

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We obtained IRB approval on December 7th , 2009. We applied for and received a COC from the NIH as required and received final approval from our IRB on March 25th 2010. The test material DHA and the placebo were acquired from Martek with a significant and unforeseen delay on August 20th 2010 (please see task #2). The Data Safety Monitoring Board and all other requirements for subject recruitment have been completed. Additional sources of subjects are being arranged to make up for delays occurring in obtaining IRB approval and issues obtaining the material from Martek (please see Partnering project W81XWH-08-1-0730, Tasks #1 and #2). A tissue bank has been applied for and approved as per our IRBs requests.
Task #4 & #7 & #8 has been accomplished. Task #6 & #9 & #10 have been accomplished for the "AGRE samples Task #11 & #12 have begun for the AGRE samples.
The SOW was updated on March 22nd 2010.
Please see partnering projects W81XWH-08-1-0729 and W81XWH-08-1-0730.

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Table of Contents

Introduction:

This project is to test to see if DHA treatment can beneficially affect excretion of urinary biomarkers of oxidative stress and the autism clinical phenotype. In addition polymorphic variants of genes of certain enzymes that synthesize and metabolize docosahexaenoic acid (DHA) may contribute to the phenotype of some autism cases. We will test to see if any of these genes are risk factors for autism. We will also measure changes in excretion of the polyunsaturated fatty acid (PUFA) derived biomarkers of oxidative stress (isoprostanes and neuroprostanes) together with the changes in production of anti-inflammatory lipid mediators. We will test these biomarkers to see if we can monitor and validate effectiveness of DHA therapy. We will also test the genotypes of key DHA-metabolizing enzymes can predict which patients will respond to therapy. Please see partnering projects W81XWH-08-1-0729 and W81XWH-08-1-0730.

Body:

Project 3: PI William Johnson, MD, Initiating PI, W81XWH-08-1-0728
Please see partnering projects W81XWH-08-1-0729 and W81XWH-08-1-0730.

Task #1 Obtain IRB approval (first 4-6 months of 01 year, W. Johnson).

This task has been accomplished.

The months of October and November were used to answer additional IRB issues. We obtained IRB approval on December 7th 2009 with the stipulation that we apply to the NIH for a Certificate of Confidentiality (COC) and if/when we received it we would have to send in an amendment to our IRB. We sent the approved protocol and related documents to the Human Research Protection Office (HRPO), Office of Research Protections (ORP) of the DOD for review on December 10th 2009. We applied for the COC on December 15th, 2009. We received a COC March 2nd 2010. The COC and the requested changes received from the Human Research Protection Office (HRPO), Office of Research Protections (ORP) of the DOD on January 9th 2010 were submitted as an amendment to our IRB office on March 8th. This went for an expedited review and we were informed on March 25th that the amendment was accepted. All relevant documents were sent to the Human Research Protection Office (HRPO), Office of Research Protections (ORP) of the DOD on March 30th.

As per our IRB's request we submitted an application for a tissue bank in Y01. After IRB review we made requested changes and sent in our replies. A second review and several meetings with the IRB resulted in a major re-design of the tissue bank. During Y02 we continued the work of establishing a tissue bank. We submitted the re-designed bank to the IRB on December 17th, 2009. We received the review of the submission on January 21, 2010 with additional requirements and considerations. From this point on a meeting was set up with the IRB to review the IRB response and a second meeting to review our answers before submission. We responded to the IRB's suggestions and requirements on May 26, 2010. We received a response on June 18, 2010. Our response was sent in on July 15, 2010. We received a response on July 26, 2010. Our response was submitted on August 24th 2010. Additional changes were made during a meeting with the IRB on September 17ht 2010.   We have been notified that the Tissue Bank has been approved and we should be receiving the approval documents within 1 – 2 weeks. Once we have received we will begin the process of amending the protocol and consents/assent to allow future use of samples so that they can be used for future studies based on the data obtained from this project.

As originally designed samples from AGRE would be obtained by this project (W81XWH-08-1-0728 Initiating Project). To simplify matters since the IRB approval is in Dr. Novotny's name this task was moved to Partnering Project W81XWH-08-1-0730. In brief a material transfer agreement and a formal application including the changes in the research design made in the last two quarters for this project (SNP and trio selection using the GWAS data) to AGRE. The application was reviewed and accepted.

Due to the fact that the IRB process took longer then expected the SOW was updated on March 22nd 2010.

Task #2 Obtain blood samples from 66 DHA-treated and 66 placebo-treated autism cases (total 132) from Project #1 (6-30 months, E. Stenroos).

An unanticipated problem in subject recruitment was getting the DHA and Placebo from Martek. We submitted the fully approved protocol to the supplier of the DHA and Placebo Martek along with all relevant documents and medical licenses and a completed FDA form 1572 on April 1st 2010 as required by Martek to initiate a materials transfer agreement. On April 15th 2010 we received an e-mail from a Martek representative. They requested that we change our IRB protocol by significantly increasing the dosage to "maximize the chances of success". We strongly disagreed with their suggestion and prepared and sent a response. The response was based on studies showing that too large a dose could change normal processing of DHA as well as excretion of biomarkers. A telemeeting was held between a representative from Martek and Mr. Stenroos on April 23d 2010. It was then agreed that our protocol was correct and acceptable. Martek then went to work on the material transfer agreement. We received the agreement on May 19th 2010. Upon review there were two items of concern. 1.) Martek was asking for all patent rights on the project. 2.) Martek was asking the University and therefore the State of NJ to take responsibility for insurance costs if there was an adverse effect. Naturally, neither of these items is acceptable to the University. We had a meeting with our lawyer responsible for negotiating this on May 27th 2010 and a follow up meeting to finalize the response on June 9th 2010. Our licensing department sent a reply on June 17th 2010. The response to the first item was to notify Martek what patents had previously been applied for, therefore indicating the University's ownership of Intellectual Property related to the project. In addition our licensing department suggested that all intellectual property solely developed by Martek should be patented by Martek, all intellectual property solely developed by us should be patented by us and all intellectual property developed together should be shared equally. The response to the second item was to notify Martek as to what is legal and acceptable to UMDNJ and the State of NJ. We received a response from Martek on July 20th . We received the final Materials Transfer agreement from Martek on August 4th . We returned the signed copy to Martek on August 9th. We received the DHA and Placebo August 20th.

Additional items done in-order to start subject recruitment in brief (please see Task#2 of project W81XWH-08-1-0730 for details).
A Continuing review was submitted. Lists of subjects interested in participating in studies have been organized by contacting special schools, pediatricians, psychiatrists and special educators in the Middlesex County, where we are based, as well as the neighboring counties. Letters to these professionals have helped in bringing forward many potential subjects who have been contacting Dr. Novotny**.** Recruitment letters have been written and are waiting IRB approval for both Dr. Lambert and Dr. Ming. Once approved they will send the letters to their subjects,

6

recruited for pervious projects that expressed a wish to be informed about future projects. Subjects from these two projects have already undergone ADOS and ADI and so will save us time in recruitment. In addition, we are in the process of arranging additional sources of ADOS and ADI tested subjects so that we can reach our recruitment goals more quickly. The Data and Safety Monitoring Board (DSMB) has been set up. The members are Dr's Wei-Ting Hwang of UPenn, Kapila Seshadri of UMDNJ-RWJ and Bart Kamen of UMDNJ-RWJMS. The initial meeting of the DSMB will be held soon. This is to be done prior to the initiation of the trial as per the protocol.

Once the board has met and given approval subject recruitment will begin.

Blood samples will be obtained as subjects are recruited, drawn, aliquoted and stored until enrollment is complete.

Task #3 Aliquot blood samples, store them in the Laboratory of Molecular Neurogenetics freezers (6-30 months, E. Stenroos).

The samples will be aliquoted as we obtain the samples and stored until subject recruitment, enrollment and treatment have been completed.

Task #4 Receive & organize DNA samples from the AGRE Repository (01 year, E. Stenroos).
This task has been accomplished.

As originally designed this project called for the testing 593 AGRE trios with subjects being diagnosed with autism by both ADOS and ADI. A change was made to take advantage of already available data.

In Y02 while finalizing IRB approval we decided to take advantage of data that had already been generated on the same samples that we are planning on using. One of the conditions of use of the AGRE samples is that data generated from the AGRE samples can be made available to those that have applied to AGRE. There are several available datasets generated with the AGRE samples. Two of these are useful to this project, one using 777 AGRE families on the Affymetrix Mapping 500K Set contributed by the Autism Consortium and the other using 943 AGRE families on the Illumina HumanHap550 chip contributed by Children's Hospital of Philadelphia. Upon investigation we found that a large number of trios had been typed with both arrays. While both arrays genotyped SNP's within the candidate genes neither had sufficient coverage to have a high likelihood of finding and association if it existed and many of the SNP's used were not useful. Conversely, both arrays genotyped some very useful haplotypes tagged SNP's within the candidate genes. So rather then re-genotype the same trios for the same useful SNP's that had been typed by these two previous projects we decided to use their data to select additional SNP's to be typed to have a much better chance of finding an association if present (please see task #8).

In order to utilize the previously generated GWAS data we needed to determine how many trios were typed in both datasets that meet our criteria. To do this required three things.
1) Determining which families were in both GWAS studies. Starting with the two datasets we began generating a data file with the necessary data for families that were typed in

both GWAS studies. Unfortunately the family data from both GWAS studies had been re-coded (the subjects ID numbers and Family ID numbers had been changed and/or truncated). We received a key from AGRE but while the individuals can be identified due to change in case and truncation there is no one field that is the same for the datasets and the key and we therefore had to make the changes and merge the datasets mostly manually.

2) Select affected within the families. Many of the AGRE families have more then one affected individual. Our study will use only Parent Child Trios. We therefore needed a principled way to chose which affected subject to genotype. We decided to choose the first affected selected individual that met our phenotype criteria. We think this is the best way to generate a dataset that would reflect a dataset collected as trios.

3) Merge data with phenotype data from AGRE. The datasets from both studies have a single field for phenotype (y or n). In addition both studies used different criteria to decide which subjects are affected. We therefore needed to merge the raw phenotype data we received from AGRE with our data file so that we can select the individuals phenotype based on our criteria.

In addition we decided to have the best chance of success we needed to limit the data to Non-Hispanic White and to exclude those that have race and/or ethnicity "unknown".

We found that 712 families were typed in both GWAS studies, 629 of which were useful as trios. Of these 443 trios were diagnosed to have autism by both ADOS and ADI. In addition, about 269 trios have been diagnosed to have autism by ADOS but either were not tested with ADI or had an ADI score that led to a diagnosis of NQA. For those not tested for ADI we will monitor the AGRE updates and add trios where ADI has been newly done and a diagnosis of autism is obtained or updated.

The samples from AGRE are at the Bionomics Research and Technology Center of UMDNJ-RWJ /Rutgers (SNP High Throughput Facility) and are ready for genotyping.

Task #5 Extract blood DNA from 132 new autism cases, 99 Neurogenetics autism trios (01 year for Neurogenetics samples, 6-30 months for 132 new autism cases, E. Stenroos).

This task will not begin until subject recruitment, enrollment and treatment have been completed.

Task #6 Organize and quantitate DNA samples from 132 new autism cases, 99 autism Neurogenetics trios & 592 AGRE trios, organize them for genotyping (01 year for Neurogenetics & AGRE samples, 6-30 months for new autism cases, E. Stenroos).
The samples from AGRE are at the Bionomics Research and Technology Center of UMDNJ-RWJ /Rutgers (SNP High Throughput Facility) and are ready for genotyping.

This task will not begin for the 132 study subjects and 99 neurogenetics samples until subject recruitment, enrollment and treatment have been completed.

Task #7 Obtain & organize primers for full GSTM1*0 genotyping (01 year, E. Stenroos).
This task has been accomplished.

Task #8 Select, obtain & organize primers for SNPstream genotyping (01 year, E. Stenroos).
This task has been accomplished.
Primers and probes have been synthesized.

Two changes were made to the original project design to take advantage of newly available technologies and data.
First we are taking advantage of new technologies that have become available to us since the start of this project. We are changing platforms for genotyping the candidate SNPs from SNPStream to the Fluidigm Dynamic Array. The Fluidigm system gives us a high throughput highly reproducible system that will help us complete genotyping in a shorter amount of time.

Second we are taking advantage of previously generated data. As originally designed this project called for the testing of 103 polymorphisms picked by the software tool Tagger for 12 candidate genes. The polymorphisms selected represent as many different haplotypes as possible for each gene. To maximize our chance of success we used the SNP's genotyped in the two previously mentioned GWAS datasets (please see task #4) to chose what additional SNP's needed to be genotyped. These GWAS SNP's were entered into the software Tagger. To do this we utilized the "Map files" for the arrays used which are freely available from Affymetrix and Illumina. These files contain the SNPs and their map coordinates as per NCBI Build 36 for most SNP's and Map Build 35 for some. We used the "liftover tool", (University of California, Santa Cruz) to translate the NCBI build 36 map coordinates to NCBI Build 35. This is necessary since Tagger, (The Broad Institute, Cambridge, MA.), the software used to chose the tagged SNPs only works with Build 35. For each candidate gene the translated coordinates entered into the Tagger program along with a list of SNPs already typed for that gene on both arrays using target criteria of r^2 > .8 for SNPs with minor allele frequency > 5%. For each gene and SNP the following were noted. 1) Which strand the gene is on (+ strand or -), 2) Which strand the SNP is on, and 3) If the SNP is exonic, intronic, UTR or in the flanking sequence. 4) If the SNP is functional. All SNPs were then checked vs. the NCBI database "SNP" as well as the Genome Variation Server (GVS) (University of Washington) to insure that they were in the correct locations of the correct candidate genes. Our dataset will therefore initially consist of 215 SNP's, 96 typed by us. These 96 SNP's represents a majority of SNP typing on the AGRE samples. If an association is found we will type additional SNP's within those regions.

Task #9 Prepare samples for GSTM1*0 Real Time PCR genotyping (6-30 months, E. Stenroos).
The samples from AGRE are at the Bionomics Research and Technology Center of UMDNJ-RWJ /Rutgers (SNP High Throughput Facility) and are ready for genotyping.

This task will not begin for the 132 study subjects until subject recruitment, enrollment and treatment have been completed.

Task #10 Prepare samples for SNPstream genotyping (6-30 months, E. Stenroos).
The samples from AGRE are at the Bionomics Research and Technology Center of UMDNJ-RWJ /Rutgers (SNP High Throughput Facility) and are ready for genotyping.

This task will not begin for the 132 study subjects until subject recruitment, enrollment and treatment have been completed.

Task #11 Genotype samples for GSTM1*0 using Real Time PCR (6-30 months, E. Stenroos, L. Shein, RWJMS DNA Core).

This task has begun for the AGRE samples.

This task will not begin for the 132 study subjects until subject recruitment, enrollment and treatment have been completed.

Task #12 Genotype SNPs of genes related to DHA metabolism using SNPstream: AGRE trios, Neurogenetics trios & new autism cases (6-30 months, E. Stenroos & A. Brooks).

This task is ready to start for the AGRE samples and will begin as soon as the assays have been tested. We have switched platforms from SNPstream to the Fluidigm Dynamic Array system. We expect this will reduce genotyping time significantly.

This task will not begin for the 132 study subjects until subject recruitment, enrollment and treatment have been completed.

Task #13 Organize, clean & analyze GSTM1*0 genotypes from the 132 new autism cases and correlate them with level of isoprostane excretion, levels of excretion of other biomarkers of oxidative stress and clinical response to treatment (6-36 months, S. Buyske & E. Stenroos).

This task will begin when all relevant data have been obtained. Note, isoprostane excretion data will be from the partnering project W81XWH-08-1-0729.

Task #14 Organize, clean and analyze genotype data from DHA metabolism genes from autism trios (AGRE & Neurogenetics) and from 132 new autism cases and correlate these genotypes with autism, level of isoprostane excretion, levels of excretion of other biomarkers of oxidative stress and clinical response to treatment (6-30 months, S. Buyske & E. Stenroos).

This task will begin when all relevant data have been obtained. Note, isoprostane excretion data will be from the partnering project W81XWH-08-1-0729.

Task #15 Manuscripts prepared and submitted for publication (03 year, all investigators).

This task will begin once all data have been analyzed.

Key Research Accomplishments

There are no Key Research Accomplishments yet.

Reportable Outcomes:

There are currently no reportable outcomes.

Conclusion:

We obtained IRB approval on December 7th , 2009. We applied for and received a COC from the NIH as required and received final approval from our IRB on March 25th 2010. We added the use of data from previously completed GWAS studies to augment the genotyping we do on the candidate genes to give us a better chance of finding an association if one exists. We have completed or made good progress on several tasks. We have completed the process of choosing SNP's and trios from AGRE. The AGRE samples are prepared and ready for genotyping. Genotyping on GSTM1 has begun on the AGRE trios and will begin shortly on the candidate genes. We changed genotyping platforms and expect to be able to complete a large percent of the genotyping rather quickly. Our tissue bank has been approved and we will amend the protocol to allow placing the samples in the bank so that they can be used for follow up studies.

Please see partnering projects W81XWH-08-1-0729 and W81XWH-08-1-0730.

References:

There are no references.

Appendices:

There are no appendices.